IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No.	:	10/549,389
Applicant	:	Shigeru KANAOKA
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For	:	METHOD OF DETECTING COLON CANCER MARKER
Art Unit	:	1637
Examiner	:	Suchira PANDE
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DECLARATION OF ACCURACY OF TRANSLATION
(37 C.F.R. 1.55)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

MAIL STOP AMENDMENT

S I R :

The undersigned translator, having an office at

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states that:

(1) I am fully conversant both with the Japanese and English languages.

(2) I have translated into English Japanese Patent Application Number JP 2003-75552, filed March 19, 2003. A copy of said English translation is attached hereto.

(3) The translation is, to the best of my knowledge and belief, an accurate translation from the original into the English language.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: April 26. 2007 Otoya Suzuki

Otoya Suzuki
(Type name of translator above)

SPECIFICATION

METHOD OF DETECTING COLON CANCER MARKER

CLAIMS

1. A method for preparing a sample to extract RNA used in a tumor marker detecting method for diagnosing colon cancer, comprising the following process;

a) a process to freeze immediately a collected biological sample by using liquid nitrogen;

b) a process to homogenize the frozen sample in the presence of an RNase inhibitor, to prepare a suspension thereof;

c) a process to extract RNA from the obtained suspension;

d) a process to reverse transcribe the extracted RNA to give cDNA;

e) a process to amplify the obtained cDNA; and

f) a process to detect the amplified cDNA,

characterized by involving no procedure of separating cell components from the biological sample.

2. A method according to claim 1, wherein the RNase inhibitor is guanidine thiocyanate.

3. A method according to claim 1 or 2, wherein the biological sample is feces.

4. A method according to any one of claim 1 to 3, wherein the tumor marker is COX-2.

5. A kit for preparing a sample to extract RNA used in a tumor marker detecting method for diagnosing colon cancer, comprising the following means;

a) a means to freeze immediately a collected biological sample by using liquid nitrogen;

b) a means to homogenize the frozen sample in the presence of an RNase inhibitor, to prepare a suspension thereof;

c) a means to extract RNA from the obtained suspension;

d) a means to reverse transcribe the extracted RNA to give

cDNA;

e) a means to amplify the obtained cDNA; and

f) a means to detect the amplified cDNA,

characterized by involving no means for separating cell components from the biological sample.

[0001]

Technical field of the invention

The present invention relates a method for preparing a sample to extract RNA used in a tumor marker detecting method for diagnosing colon cancer, comprising the following process;

a) a process to freeze immediately a collected biological sample by using liquid nitrogen;

b) a process to homogenize the frozen sample in the presence of an RNase inhibitor, to prepare a suspension thereof;

c) a process to extract RNA from the obtained suspension;

d) a process to reverse transcribe the extracted RNA to give cDNA;

e) a process to amplify the obtained cDNA; and

f) a process to detect the amplified cDNA,

characterized by involving no procedure of separating cell components from the biological sample.

[0002]

Background of the invention

The deaths by colon cancer are increasing. The number of deaths by colon cancer is the fourth large among male, and the second large among female deaths in all cancer deaths (Statistics of Japanese cancer deaths in 1999). According to an estimation of cancer patients in 2015 in Japan, number of colon cancer patients is estimated to be the first in both male and female. Global measures to counter colon cancer including secondary prevention are thus required, and mass screening of cancer may be one of the most effective methods.

[0003]

For the mass screening of cancer, it is important that the detection method is easy and non-invasive. The only non-invasive method now available is the method to examine existence of occult blood in feces, that is, the fecal occult blood test, and is used extensively as a

standard method of the mass screening of colon cancer.
[0004]

However, the fecal occult blood test has rather low sensitivity and specificity (the sensitivity: 60 %, the specificity: 45%), because appearance of hemoglobin in feces is not specific to tumor. Further, there is a shortcoming that false negatives and false positives exist.
[0005]

Also, in the diagnosis of colon cancer, after or in parallel with the screening by the immunological fecal occult blood test, total colonoscopy or a combination of Ba-enema and sigmoidoscopy has been adopted. There is thus a shortcoming that it needs much time and effort.
[0006]

As alternative methods to the fecal occult blood test, methods using DNA are reported, such as detection of mutations in K-ras, p-53, or APC genes, or detection of microsatellite instability in feces (Nonpatent Literatures 1-4).
[0007]

These methods using DNA are non-invasive and can capture the direct changes in cancer cells, and have characteristics of having high specificity, and so are considered to be a hopeful method in the future. However, it has a demerit that the sensitivity is lower compared to the fecal occult blood test, a prior art, and is rather time and effort-consuming.
[0008]

Further, as an alternative method to the fecal occult blood test, in order to detect gene expression more directly, a method for detecting mRNA of protein kinase C (PKC) or the like in the feces has been developed (Nonpatent Literatures 5-7).
[0009]

However, the method making use of RNA described above could not have the sensitivity exceeding that of the fecal

occult blood test method, because it was impossible to extract RNA easily and efficiently from a small amount of feces.

[0010]

A method to detect RNA qualitatively and quantitatively by combining the PCR method with the reverse transcriptase reaction (RT), has been known. This RT-PCR method is superior to Northern blot technique in the high sensitivity to be able to detect trace molecules, and is more advantageous than the in situ hybridization technique in speed and easiness of manipulation.

[0011]

However, since RNA is more unstable compared with DNA and is always subjected to a danger of decomposition by RNA digesting enzymes (RNases) which are ubiquitous in all the biological samples and very stable, strict control to avoid contamination of RNases is necessary in the RT-PCR method, during and after purification processes of RNA.

[0012]

Therefore, when RNA is extracted from the feces, which is a biologically very crude sample, a process to separate the cell fraction in advance has been necessary, to exclude effects of RNases.

[0013]

Accordingly, it has been considered impossible to extract RNA directly from feces containing enormous amount of RNases derived from very large amount of microorganisms, and, a separation of the cell fraction has been considered to be essential for removing at least exogenous RNases derived from microorganisms or the like.

[0014]

Surprisingly, however, the inventor of the present invention found that homogenization of frozen biological materials in the presence of RNase inhibitors can resolve the problems described above, and has completed the present invention.

[0015]

[Nonpatent Literature 1]

D. Sidransky, et al., Science, 256, April 3,1992, 102-105;

[Nonpatent Literature 2]

S. M. Dong, et al., Journal of the National Cancer Institute, 93 (11), June 11, 2001, 858-865;

[Nonpatent Literature 3]

G. Traverso, et al., The New England Journal of Medicine, 346 (5), January 31, 2002, 311-320;

[Nonpatent Literature 4]

G. Traverso, et al., The Lancet, 359, February 2, 2002, 403-404)

[Nonpatent Literature 5]

L. A. Davidson, et al., Carcinogenesis, 19(2), 1998, 253-257;

[Nonpatent Literature 6]

R. J. Alexander and R. F. Raicht, Digestive Diseases and Sciences, 43(12), 1998, 2652-2658;

[Nonpatent Literature 7]

T. Yamao, et al., Gastroenterology, 114(6), 1998, 1198-1205

[0016]

Problem to be solved by the invention

It is therefore an object of the present invention to provide a non-invasive and convenient tumor marker detecting method for diagnosing colon cancer, which is superior in sensitivity and specificity to the existing fecal occult blood test.

[0017]

Solution for solving the problem

The object of the present invention is to provide a method for preparing a sample to extract RNA used in a tumor marker detecting method for diagnosing colon cancer, comprising the following process;

a) a process to freeze immediately a collected biological sample by using liquid nitrogen;

b) a process to homogenize the frozen sample in the presence of an RNase inhibitor, to prepare a suspension thereof;

c) a process to extract RNA from the obtained suspension;

d) a process to reverse transcribe the extracted RNA to give cDNA;

e) a process to amplify the obtained cDNA; and

f) a process to detect the amplified cDNA,

characterized by involving no procedure of separating cell components from the biological sample.

[0018]

As for the RNase inhibitors of the present invention, guanidine thiocyanate, Isogene, Ultraspec II (a registered trade mark) and the like are included.

[0019]

The biological samples of the present invention are tissues of animals and plants, body fluids, excrements and the like, and preferably are feces, and more preferably are human feces.

[0020]

The frozen sample may be preserved in frozen state. The preservation temperature is -75 to -196°C, preferably -110 to -196°C, and more preferably -196°C. The preservation period is one day to 10 years, preferably one day to 3 years, and more preferably one day to one year.

[0021]

The tumor marker used in the present invention is COX-2, matrixmetalloprotease (MMP), c-met, CD44, EGF-R, EF-1, Wnt-2, and is preferably COX-2.

[0022]

The processes c) to e) described above are called as the RT-PCR method, and can be carried out, for example, according to the description by T. Sekiya et al. eds., Forefront of PCR method, 1997, Kyoritu Pub., 187-196.

[0023]

Extraction of RNA from the suspension can be carried

out using methods well known in the art, and using commercially available kits, for example, RNeasy Mini (QIAGEN) or RNA Extraction Kit (Pharmacia Biotech).

[0024]

"Reverse transcription" in the present invention means conversion of RNA to the complementary DNA (cDNA) using a reverse transcriptase. The reverse transcription reaction is usually conducted using a solution containing a buffer, salts such as $MgCl_2$, KCl, and the like, dithiothreitol (DTT), a primer, kinds of deoxyribonucleotides, RNase inhibitors, and a reverse transcriptase. The salts described above can be appropriately replaced by other salts after testing. Proteins such as gelatine, albumin or the like, or detergents can also be added.

[0025]

For amplification of cDNA carried out subsequent to the reverse transcription, the PCR technique is usually adopted. The PCR reaction mixture usually contains a buffer, salts such as $MgCl_2$ and KCl, primers, kinds of deoxyribonucleotides, and a heat resistant polymerase. The salts described above can be appropriately replaced by other salts after testing. Proteins such as gelatine, albumin or the like, dimethylsulphoxide, detergents, or the like, can also be added.

[0026]

For amplification of cDNA, the LAMP method (Japan Patent No.3313358) or the ICAN method (Japan Patent Laid-Open No.2001-136965) can be used.

[0027]

A "primer" in the present invention means an oligonucleotide which works as a synthesis initiation point in the case of cDNA synthesis or polynucleotide amplification. The primer is preferably a single-strand, but a double-strand can also be used. When the primer is a double-strand, it is preferable to make it single-

stranded prior to the amplification reaction. The primer can be synthesized according to a method well known in the art, or can be isolated from the living organisms.

[0028]

The reverse transcriptase used in the reverse transcription reaction is an enzyme capable of reverse transcribing RNA to cDNA. As for the reverse transcriptase, there are reverse transcriptases derived from retroviruses such as RAV (Rous associated virus), AMV (Avian myeloblastosis virus) and the like, and reverse transcriptases derived from mouse retroviruses such as MMLV (Moloney murine leukemia virus) and the like, but it is not limited to the aboves.

[0029]

As the heat resistant polymerase used for PCR, Taq polymerase can be nominated, but it is not confined to this.

[0030]

As the detection method of amplified DNA, electrophoresis using agarose gel can be used, but the method may not be confined to this.

[0031]

Further, another object of the present invention is to provide a kit for preparing a sample to extract RNA used in a tumor marker detecting method for diagnosing colon cancer, comprising the following means;

a) a means to freeze immediately a collected biological sample by using liquid nitrogen;

b) a means to homogenize the frozen sample in the presence of an RNase inhibitor, to prepare a suspension thereof;

c) a means to extract RNA from the obtained suspension;

d) a means to reverse transcribe the extracted RNA to give cDNA;

e) a means to amplify the obtained cDNA; and

f) a means to detect the amplified cDNA,

characterized by involving no means for separating cell components from the biological sample.

[0032]

Further, the kit according to the present invention may contain an instruction describing the methods of the present invention.

[0033]

Example 1

The following examples illustrate the present invention, but do not limit the invention.

[0034]

Among patients hospitalized in the First Department of Internal Medicine of Hamamatsu University School of Medicine for detailed examination and therapy, 30 cases confirmed to have colon cancer and 22 cases to have no tumor or inflammatory alteration in their colon (non colon disorder) by the total colonoscopy were selected as the subject of the study. Informed consents of all the cases had been obtained.

[0035]

As soon as possible after sampling feces, the feces were separated into 5 ml tubes about 1 g each, were frozen using liquid nitrogen, and were stored at -80°C. Also, for comparison and reference, human hemoglobin (Hb) in the feces of each sample was measured by the immunological fecal occult blood test. Tissue biopsy specimen, taken both from the cancer and the normal parts when the endoscopy was carried out before the therapy, were frozen by liquid nitrogen and stored at -80°C. Then, feces were homogenized using a homogenizer, guanidine salt, and phenol, and whole RNA was extracted using chloroform and ethanol.

[0036]

One μg of the obtained RNA was reverse transcribed using ReverScript II (a registered trade mark), (reaction

mixture volume: 20 µl, Wako Pure Chemical Industries) to give cDNA. A part thereof was amplified by means of nested PCR using GeneTaq (Wako). The PCR product obtained was electrophresed on 4% agarose gel, and stained by ethidium bromide.

[0037]

Here, the primers used were: the random primers in reverse transcription, and in PCR, were those reported by Gerhard (JJCO, 1994) for CEA, and were originally designed for COX-2. The first round of PCR was executed 20 cycles, and the second round 25 cycles.

[0038]

The followings indicate the primers used.

<CEA>

[Table 1]

Forward 1: 5'-TCTGGAACTTCTCCTGGTCTCTCAGCTGG-3'

Forward 2: 5'-GGGCCACTGCTGGCATCATGATTG-3'

Reverse: 5'-TGTAGCTGTTGCAAATGCTTTAAGGAAGAAGC-3'

<COX-2>

[Table 2]

Forward 1: 5'-CTGAAAACTCCAAACACAG-3'

Forward 2: 5'-GCACTACATACTTACCCACTTCAA-3'

Reverse: 5'-ATAGGAGAGGTTAGAGAAGGCT-3'

[0039]

Results

Feces from 30 colon cancer cases (3 early cancer and 27 advanced cancer cases) and from 22 non colon cancer cases were examined by RT-PCR, in order to detect CEA and COX-2, and the following results were obtained.

[0040]

CEA was detected in all cases among the 30 colon cancer cases, and in 21 among 22 non colon cancer cases. Also, it turned out that RNA suitable for RT-PCR amplification could be extracted from both samples.

[0041]

COX-2 was detected in 27 cases among the 30 colon cancer cases (caeca: 2/2, ascending colon: 3/5, descending colon: 1/1, sigmoid colon: 7/7, rectum: 12/13; early cancer: 2/3, advanced cancer: 25/27), but was not detected in any of 22 non colon cancer cases (sensitivity: 90%, specificity: 100%).

[0042]

Human hemoglobin in feces (Hb) by using the immunological fecal occult blood test, 23 among 28 colon cancer cases and 3 among 22 non colon cancer cases were positive (sensitivity: 82.1%, specificity: 86.3%).

[0043]

Among three COX-2 negative colon cancer cases, one was positive in the immunological fecal occult blood test, and 2 were negative.

[0044]

COX-2 was detected in 3 among 5 colon cancer cases negative to the immunological fecal occult blood test,

[0045]

Example 2

The amount—and the distribution of molecular weights of total RNA obtained from human feces according to the method of the present invention were compared with those obtained according to Alexander's method (R. J. Alexander and R. F. Raicht, Digestive Diseases and Sciences, 43(12), 1998, 2652-2658). As a control, total RNA was extracted from the human colon cancer tissues using a commercially available RNA extraction reagent (ISOGEN, Wako)

[0046]

The same amount of total RNA extracted from each sample was electrophoresed on an agarose gel.

[0047]

Two main bands recognized on the lane 3 (RNA derived from human colon cancer tissues) show 28s and 18s rRNAs. Smeared parts thereon indicate that various kinds of high

molecular weight RNAs are contained in the obtained total RNA.

[0048]

Two main bands recognized on the lane 2 (RNA derived from feces obtained by the method of the present invention) show 23s and 16s rRNAs derived from enteric bacteria. Since smeared parts were also recognized thereon similarly to the lane 3, the total RNA obtained from the feces by the method of the present invention is considered to contain also various kinds of high molecular weight RNAs.

[0049]

Contrarily, any bands and smears were not detected at all in the lane 1, showing that high molecular weight RNAs were not contained in the extract of the sample.

In fact, the desired products were obtained from the sample of lane 2 by the RT-PCR technique, but no PCR products were obtained from the sample of lane 1.

[0050]

From the results of the present studies, it became obvious that the RNA extracted from the human feces by the method of the present invention can be amplified by means of the RT-PCR technique. Also, the detection of COX-2 from the feces by the RT-PCR technique had 90% sensitivity and 100% specificity, and it is proved that the present invention is superior to a conventional technique of the immunological fecal occult blood test.

[0051]

Further, since the method of the present invention needs smaller amount of feces for detection and has higher detection sensitivity compared to the detection of the gene mutation of APC, K-ras, or p53, it can save largely the time and effort needed for the detection.

[0052]

While the conventional technique of the fecal occult blood test targets a general and indirect event,

"bleeding" from the lesion, the method of the present invention targets a specific and direct event, the expression of a marker of carcinogenesis, COX-2. Therefore, the data obtained by the method of the present invention provide diagnosis with higher quality.

[0053]

Accordingly, the method of the present invention is clinically very useful as a novel non-invasive screening method with high specificity and high sensitivity.

Brief description of drawings

[0054]

SEQUENCE LISTING

<110> Shizuoka Technology Licensing Organization

<120> Detection method of tumor marker for colon cancer diagnosis.

<130> KP-10665

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<170> PatentIn version 3.1

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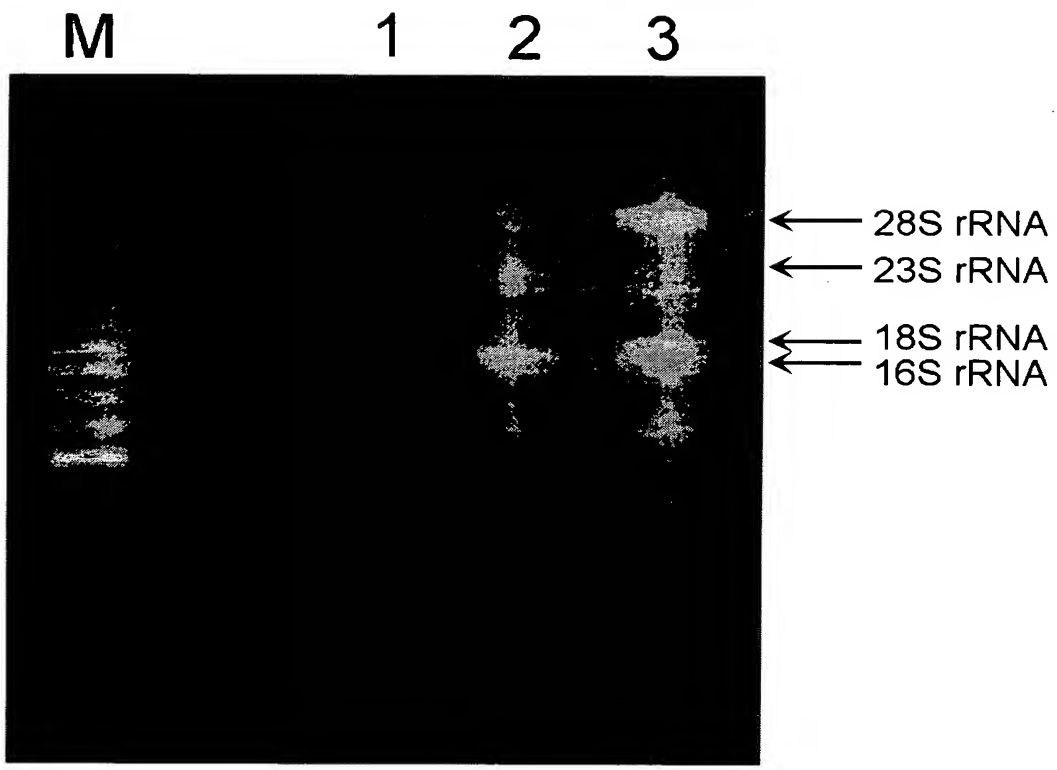
Brief description of figures

[Fig. 1]

Fig. 1 shows a result of an electrophoresis in Example 2. The lane 1 shows total RNA extracted from human feces with the method by Alexander et al. The lane 2 shows total RNA extracted from human feces with the method of the present invention. The lane 3 shows total RNA extracted from a human colon cancer tissue. The lane M shows the molecular weight markers.

1 / 1

Fig. 1



ABSTRACT

It is intended to provide a non-invasive and convenient method of detecting a tumor marker for diagnosing colon cancer which is superior in sensitivity and specificity to the existing fecal occult blood test. More specifically speaking, a method of detecting a tumor marker for diagnosing colon cancer which comprises collecting biological sample which is immediately frozen using liquid nitrogen in some cases, homogenizing the sample in the presence of an inhibitor of an RNA digesting enzyme to give a suspension, extracting RNA from the obtained suspension, subjecting the extracted RNA to reverse transcription to give cDNA, amplifying the obtained cDNA and then detecting the thus amplified cDNA. This method is characterized by involving no procedure of separating cell components from the biological sample.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Dorothy DeFrancesco

In the event that this Paper is late filed, and the necessary petition for extension of time is not filed concurrently herewith, please consider this as a Petition for the requisite extension of time, and to the extent not tendered by Form PTO-2038 attached hereto, authorization to charge the extension fee, or any other fee required in connection with this Paper to Account No. 06-1378.

AMENDMENT UNDER 37 CFR 1.111

Commissioner for Patents
P.O. Box 1450
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MAIL STOP AMENDMENT

S I R :

Responsive to the Office Action mailed January 5, 2007, the term for response to which is extended one month by a PETITION FOR EXTENSION OF TIME being filed concomitantly herewith to expire on May 5, 2007, please amend the above-identified application as follows:

Amendments to the Claims are reflected in the listing of claims which are set forth on page 2 of this paper.

Remarks begin on page 7 of this paper.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

Claim 1. (currently amended) A method for preparing a sample to extract RNA used in a tumor marker detecting method for diagnosing colon cancer[[,]] ~~comprising~~ [[the]] ~~following process~~ consisting essentially of:

a) [[a]] ~~process~~ [[to]] ~~homogenize~~ [[the]] homogenizing a collected biological sample in the presence of an RNase inhibitor[[,]] to prepare a suspension thereof[[;]], ~~characterized by involving no procedure of~~ without separating cell components from the biological sample.

Claim 2. (original) A method according to claim 1, wherein the collected biological sample is frozen.

Claim 3. (previously presented) A method according to claim 1, wherein the RNase inhibitor is guanidine thiocyanate.

Claim 4. (previously presented) A method according to claim 1, wherein the biological sample is feces.

Claim 5. (currently amended) A tumor marker detecting method for diagnosing colon cancer[[,]] ~~comprising the following processes~~ consisting essentially of:

a) homogenizing a collected biological sample in the presence of an RNase inhibitor to prepare a suspension, without separating cell components from the biological sample;

b) [[a]] ~~process to extract~~ extracting RNA from the ~~obtained~~ sample [[for]] ~~extracting~~ obtained from step a) to provide extracted RNA;

c) [[a]] ~~process to~~ carrying out reverse ~~transcribe~~ transcription on the extracted RNA from step b) to ~~give~~ provide cDNA;

d) [[a]] ~~process to amplify~~ amplifying the ~~obtained~~ cDNA from step c); and

e) [[a]] ~~process to detect~~ detecting the amplified cDNA[[,]] ~~in addition~~ [[to]] ~~the method according~~ [[to]] ~~claim~~ [[1]] from step (d).

Claim 6. (previously presented) A method according to claim 1, wherein the tumor marker is COX-2.

Claim 7. (withdrawn) A kit for preparing a sample to extract RNA used in a tumor marker detecting method for diagnosing colon cancer, comprising the following means:
a) a means to homogenize a collected biological sample in the presence of an RNase inhibitor, and prepare a suspension thereof; characterized by involving no means for separating cell components from the biological sample.

Claim 8. (withdrawn) A kit according to claim 7, further comprising a means to freeze the collected biological sample.

Claim 9. (withdrawn) A kit according to claim 7, wherein the RNase inhibitor is guanidine thiocyanate.

Claim 10. (withdrawn) A kit according to claim 7, wherein the biological sample comprises feces.

Claim 11. (withdrawn) A tumor marker detecting kit for diagnosing colon cancer, comprising the following means:
b) a means to extract RNA from the obtained sample for extracting RNA;
c) a means to reverse transcribe the extracted RNA to give cDNA;
d) a means to amplify the obtained cDNA; and
e) a means to detect the amplified cDNA.

Claim 12. (withdrawn) A kit according to claim 7, wherein the tumor marker is COX-2.

Claim 13. (new) The method according to claim 1, wherein the biological sample comprises microorganisms.

Claim 14. (new) The method according to claim 5, wherein in step b) whole RNA is extracted from the sample obtained from step a) without separating RNA derived from human cells from RNA derived from bacteria.

Claim 15. (new) The method according to claim 5, wherein in step d) amplifying the cDNA from step c) is carried out by a nested PCR.

Claim 16. (new) The method according to claim 5, wherein the amplification is carried out by a PCR and a first round of the PCR is executed for 20 cycles.

Claim 17. (new) A method according to claim 5, wherein the collected biological sample is frozen.

Claim 18. (new) A method according to claim 5, wherein the RNase inhibition is guanidine thiocyanate.

Claim 19. (new) A method according to claim 5, wherein the biological sample comprises feces.

Claim 20. (new) A method according to claim 6, wherein the biological sample is frozen; the biological sample comprises feces; and the RNase inhibitor is guanidine thiocyanate.

REMARKS

The Examiner is respectfully requested to acknowledge receipt of the certified copy of the priority document.

The Office Action enclosed copies of the INFORMATION DISCLOSURE STATEMENTS BY APPLICANT Form PTO/SB/08B dated September 14, 2005 and Form PTO/SB/08B dated October 24, 2005, with the Examiner's initials in the left column next to some of the cited publications, indicating that each of the initialed cited publications was considered and made of record. On the Form PTO/SB/08B dated September 14, 2005, the Examiner drew a line through the NAKAGAWA and the KANAOKA et al. publications. NAKAGAWA and KANAOKA et al. are Japanese language publications.

On the enclosed copy of the Form PTO/SB/08B dated September 14, 2005, next to the NAKAGAWA publication, the following was typed: "English translation not provided," and next to the KANAOKA et al. publication, the following was typed: "Translation provided does not have complete citation and it is not certified."

For the following reasons, it is respectfully submitted that the Examiner return to the undersigned a copy of the Form

PTO/SB/08B dated September 14, 2005, with the Examiner's initials next to each of the cited publications, including the NAKAGAWA and KANAOKA et al. publications, to indicate that all the cited publications were considered and made of record.

The NAKAGAWA and KANAOKA et al. publications were both cited in the copy of the English-language International Search Report, which was filed on September 14, 2005. This should have been sufficient for the Examiner to consider and make of record the NAKAGAWA and KANAOKA et al. publications, as seen in the following quoted excerpt from MPEP 609.04(a)III:

> "Where the information listed is not in the English language, but was cited in a search report or other action by a foreign patent office in a counterpart foreign application, **the requirement for a concise explanation of relevance can be satisfied by submitting an English-language version of the search report or action which indicates the degree of relevance found by the foreign office.** This may be an explanation of which portion of the reference is particularly relevant, to which claims it applied, or merely an 'X', 'Y', or 'A' indication on a search report."
> [emphasis provided]

Enclosed herewith is an executed DECLARATION OF ACCURACY OF TRANSLATION FOR KANAOKA ET AL. PUBLICATION dated May 2, 2007,

which provides the certified complete citation of the KANAOKA et al. publication requested by the Examiner.

Regarding item no. 2 at the bottom of page 2 of the Office Action, under the heading "Priority," enclosed herewith is an English-language translation of applicant's priority application, along with a DECLARATION OF ACCURACY OF TRANSLATION of Otoya SUZUKI dated April 26, 2007.

Claim 1 was editorially revised and was amended to replace "comprising" with --consisting essentially of--.

Claim 5 was amended into independent form by including the features of claim 1.

New claim 13 is supported on page 3, line 17 of the specification.

New claim 14 is supported in the specification on page 8, lines 21 to 24.

New claim 15 is supported in the specification on page 8, line 23 and lines 18 to 29.

New claim 16 is supported in the specification on page 8, last two lines.

New claim 17 is supported in the specification on page 5, lines 34 to 25.

New claim 18 is supported in the specification on page 5, lines 27 to 30.

New claim 19 is supported in the specification on page 5, lines 30 to 33.

New claim 20 includes the features of claims 17 to 19.

Claims 1 to 5 were rejected under 35 USC 102 as being anticipated by Alexander and Raicht (1998), Digestive Diseases and Sciences, Vol. 43, No. 12, pp. 2652-2658, as evidenced by Ultrapsec™ -II RNA, Isolation System, Biotecx Bulletin, No. 28, 1993, for the reasons stated in item no. 5 on pages 3 to 5 of the Office Action.

The Alexander and Raicht publication is discussed on page 2, lines 19 to 33 of the present specification.

Alexander and Raicht (1998) disclose a method for preparing a sample to extract RNA comprising a step for separating cell components by using centrifugation (page 2653, left-hand column, lines 5 to 1 from the bottom).

In contrast to Alexander and Raicht, applicant's claims are directed to a method for preparing a sample to extract RNA without separating cell components by centrifugation.

Applicant's claims 1 to 5 are thus substantially different from Alexander and Raicht.

Withdrawal of the 35 USC 102 rejection is therefore respectfully requested.

Claim 6 was rejected under 35 USC 103 as being unpatentable over Alexander and Raicht (1998), Digestive Diseases and Sciences, Vol. 43, No. 12, pp. 2652-2658, as evidenced by Ultraspec™ -II RNA, Isolation system, Biotecx Bulletin, No. 28, 1993, in view of Sano et al., (1995), Cancer Research, 55: 3785-3879 for the reasons set forth in item no. 7 on pages 5 to 6 of the Office Action.

It was admitted in the Office Action that Alexander and Raicht do not teach that the tumor marker is COX-2.

Alexander and Raicht do not teach or suggest the method of applicant's claim 1. As discussed above, Alexander and Raicht do not teach or suggest that the tumor marker is COX-2.

Further, as seen in the Example 2 of the present specification, since no PCR products were obtained from the sample obtained by using the method of Alexander and Raicht comprising a step for separating cell components, the method of Alexander and Raicht is not useful for the detection of the COX-2 tumor marker (see page 10, lines 2 to 31 of the present specification).

Since it is well-known to those of ordinary skill in the art that RNA is very sensitive to RNase, it is considered that extracting RNA directly from a biological sample containing an enormous amount of RNases would not be possible and that a step for separating cell components prior to extracting RNA is essential.

Therefore, it is respectfully submitted that one of ordinary skill in the art at the time of the invention would not consider removing the step for separating cell components from the method of Alexander and Raicht.

Withdrawal of the 35 USC 103 rejection is therefore respectfully requested.

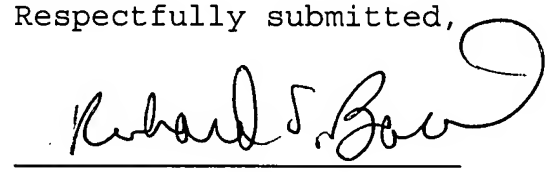
In summary, it is respectfully submitted that applicant's claims are not anticipated and are not rendered obvious over the references, either singly or combined in the manner relied upon in the Office Action, in view of the distinctions discussed hereinabove. It is furthermore submitted that there are no teachings in the references to combine them in the manner relied upon in the Office Action.

An INFORMATION DISCLOSURE STATEMENT is being filed concomitantly herewith.

Reconsideration is requested. Allowance is solicited.

If the Examiner has any comments, questions, objections or recommendations, the Examiner is invited to telephone the undersigned at the telephone number given below for prompt action.

Respectfully submitted,



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Encs.: (1) PETITION FOR EXTENSION OF TIME
(2) English-language translation of applicant's priority document, along with a DECLARATION OF ACCURACY OF TRANSLATION of Otoya SUZUKI dated April 26, 2007
(3) DECLARATION OF ACCURACY OF TRANSLATION FOR KANAOKA ET AL. PUBLICATION dated May 2, 2007
(4) INFORMATION DISCLOSURE STATEMENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No.	:	10/549,389
Applicant	:	Shigeru KANAOKA
Filed	:	September 14, 2005
For	:	METHOD OF DETECTING COLON CANCER MARKER
Art Unit	:	1637
Examiner	:	Suchira PANDE
Docket No.	:	05596/HG
Confirmation No.	:	2212
Customer No.	:	01933

DECLARATION OF ACCURACY OF TRANSLATION
FOR KANAOKA ET AL. PUBLICATION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

MAIL STOP AMENDMENT

S I R :

(1) I am fully conversant both with the Japanese and English languages.

(2) I translated from Japanese into English the English-language translation of S. KANAOKA et al., "Shou P-379 Potential of colorectal cancer screening by using RT-PCR method in which COX-2 from feces is used as a marker," JAPANESE JOURNAL OF GASTROENTEROLOGY, Vol. 99, special extra issue, 20 September 2002, page A634 Sho P-379, which was submitted with the Information Disclosure Statement filed on September 14, 2005. A

copy of said English-language translation is attached hereto.

(3) The aforesaid translation is, to the best of my knowledge and belief, an accurate translation from the original into the English language.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: May 2, 2007 By: Dorja Sugulani

Name: